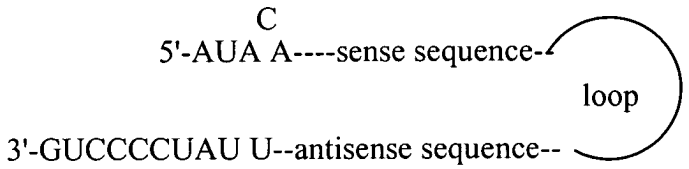
## IN THE SPECIFICATION

Please enter the substitute SEQUENCE LISTING into the specification.

Please amend the paragraph beginning on page 3, line 21, as follows:

More preferably, the sequence transcribing the pre-siRNA further comprises at the 5' and 3' termini sequences such that the transcribed pre-siRNA has the following structure:




(SEQ ID NO:1).

Please amend the paragraph beginning on page 5, line 6, as follows:

**Figure 1.** Schematic representation of the psiUX vector; sites present in the polylinker downstream for the U1 snRNA promoter are indicated. The sequence of the U1 promoter region extending from position -393 to position -6 with respect to the initiation site is shown below (SEQ IDNO:19).

Please amend the paragraph beginning on page 5, line 10, as follows:

**Figure 2.** Panel A): sequence of different psiUx derivatives and of related oligos. 5'end and 3'end of the transcripts are indicated. Sense and antisense sequences are deduced from the lamin A/C mRNA and are represented by convergent arrows. The mutations introduced in the

psiUc$_{mut}$-lam construct are indicated above the sequence. The 3' terminator sequence of the U1 gene is indicated as "3' box". Internal loops and variant sequences present in the psiUb-lam and psiUd-lam constructs are shown in grey. (SEQ ID NOs:20-25) Panel B): Predicted structures of the four tested anti-lamin primary transcripts. The arrows indicate the presumptive sites of processing by the Dicer enzyme. siRNA sequences are shown in bold and italics. Underlined nucleotides identify the sequences derived from the 5' and 3' regions of U1 snRNA. The asterisk represents the monomethyl cap. (SEQ ID NOs:26-29)

Please amend the paragraph beginning on page 6, line 14, as follows:

**Construction of psiUx vector.** The U1 snRNA gene-based vector was derived from plasmid pHU1-ID, containing the entire human gene (De Angelis et al., 2002); this plasmid carries the 600 bp *BamH*I fragment containing the transcriptional unit of the human U1 snRNA gene inserted in the *BamH*I site of the pSP65 vector (Promega) in the opposite direction of the SP6 promoter. Plasmid psiUx was derived from the latter by double digestion with *Bgl*II and *Nhe*I and religation in the presence of a polylinker containing the 5'-*Bgl*II, *Kpn*I, *Xho*I, *Nhe*I, *BamH*I, and *Nhe*I-3' sites. The *Bgl*II site maps in the U1 snRNA gene, at position -6 with respect to the transcription initiation site, while the *Nhe*I site is in the vector, 300 nucleotides upstream from the SP6 promoter. The linker was made by annealing the two oligonucleotides:
linkup: 5'- GATCTGGTACCCTCGAGGCTAGCGGATCCG-3' (SEQ ID NO:2)
linkdn: 5'- CTAGCGGATCCGCTAGCCTCGAGGGTACCA-3' (SEQ ID NO:3).

Please amend the paragraph beginning on page 7, line 1, as follows:

The selected target sequence on the lamin A/C was derived from Sui et al. (2002) and covers nucleotides 1602-1622 of the X03444 of the NCBI Database. The following oligos:
a-lamUP
5'GATCTCATACAGGGCAATTGGCAGATCAAGCGTTGTGAAGCCACAGATGAACGCT TGATCTGCCAATTGCCCTTTATCCCCTGACTTTCTGGAGTTTCAAAAGTAGAC3' (SEQ ID NO:20)

and a-lamDN

5'CTGAGTCTACTTTTGAAACTCCAGAAAGTCAGGGGATAAAGGGCAATTGGCAGAT CAAGCGTTCATCTGTGGCTTCACAACGCTTGATCTGCCAATTGCCCTGTATGA3' (SEQ ID NO:21)

were annealed and inserted in the *Bgl*II and *Xho*I sites of psiUx, giving rise to plasmid psiUa-lam. Plasmid psiUb-lam and psiUc-lam were obtained by cloning in the *Bgl*II and *Xho*I sites of psiUx the following oligos:

psiUb-lam:

b-lamUP

5'GATCTCATACAGGGCAATTGGCAGATCAAGCGTTTGTGTAGCGCTTGATCTGCCAA TTGCCCTTTATCCCCTGACTTTCTGGAGTTTCAAAAGTAGAC3' (SEQ ID NO:6)

and b-lamDN

5'TCGAGTCTACTTTTGAAACTCCAGAAAGTCAGGGGATAAAGGGCAATTGGCAGAT CAAGCGCTACACAAACGCTTGATCTGCCAATTGCCCTGTATGA3' (SEQ ID NO:7)

psiUc-lam

c-lamUP

5'GATCTCGGGCAATTGGCAGATCAAGCGTTTGTGTAGCGCTTGATCTGCCAATTGCC CTTACTTTCTGGAGTTTCAAAAGTAGAC3' (SEQ ID NO:8)

and c-lamDN

5'TCCTGAGTCTACTTTTGAAACTCCAGAAAGTAAGGGCAATTGGCAGATCAAGCGC TACACAAACGCTTGATCTGCCAATTGCCCGA3' (SEQ ID NO:9)

psiUd-lam:

d-lamUP

5'GATCTCGGGCAATTGGCAGATCAAGCGTTTGACTTCGCATGAATGAGTTCATTCAT GAAGCGAAACGCTTGATCTGCCAATTGCCCTTACTTTCTGGAGTTTCAAAAGTAGAG 3' (SEQ ID NO:10)

and d-lamDN

5'CTAGCTCTACTTTTGAAACTCCAGAAAGTAAGGGCAATTGGCAGATCAAGCGTTTC GCTTCATGAATGAACTCATTCATGCGAAGTCAAACGCTTGATCTGCCAATTGCCCGA 3' (SEQ ID NO:11)

Please amend the paragraph beginning on page 8, line 7, as follows:

Plasmid psiUc$_{mut}$-lam was obtained by cloning oligos:
cmut-lamIP
5'GATCTCGGGCAATTGcgAGATCAAGCGTTTGTGTAGCGCTTGATCTcgCAATTGCCC
TTACTTTCTGGAGTTTCAAAAGTAGAC3' (SEQ ID NO:12)
and cmut-lamDN
5'CTGAGTCTACTTTTGAAACTCCAGAAAGTAAGGGCAATTGcgAGATCAAGCGCTAC
ACAAACGCTTGATCTcgCAATTGCCCGA3' (SEQ ID NO:13)
(lower case letters indicate nucleotides mutated with respect to the lamin sequence).

Please amend the paragraph beginning on page 8, line 26, as follows:

**Northern blotting.** Total RNA isolation from transiently transfected HeLa cells was done using the Ultraspec RNA isolation system (Biotech Laboratories, Houston) according to the manufacturer's instructions. To detect siRNAs, 15 ug of total RNA were electophoresed in a 10% polyacrylamide-8 M urea gel and transferred by electroblotting onto Hybond-N$^+$ membrane (Amersham Pharmacia Biotech). The hybridisation was carried out at 37 °C in 5X SSPE, 5X Denhardt's solution, 0,5 SDS, 25 ug/ml salmon sperm DNA (Invitrogen). Washes were performed at 37 °C in 6X SSPE and 2X SSPE and 0.2X SSPE. Probes used were terminally $^{32}$P-radiolabelled DNA oligos:
probe a: 5'-GGCAATTGGCAGATCAAGCG-3'(SEQ ID NO:14);
probe a-mut: 5'-GGCAATTGCGAGATCAAGCG-3' (SEQ ID NO:15);
$\alpha$-probe: 5'- CGCTTGATCTGCCAATTGCC-3'(SEQ ID NO:16).

Please amend the paragraph beginning on page 9, line 16, as follows:

The system takes advantage of the characteristics of the human U1 snRNA gene and of its promoter and terminator regions (Hernandez, 1985; Hernandez and Weiner, 1986). The U1 promoter regulates transcription by RNA polymerase II, is ubiquitously active, and ensures high

levels of expression. The primary transcript has a monomethylated cap and the RNA is efficiently exported to the cytoplasm. This is extremely important for efficient processing of the pre-siRNA, since the Dicer enzyme has been shown to localize in the cytoplasm (Billy et al., 2001). In addition the correct 3' end formation of U1 snRNA is directed by a box element (GTTTCAAAAGTAGAC-3'box; SEQ ID NO:17) located 10 nucleotides downstream from the U1 snRNA coding region, which works only in association with the specific U1 promoter sequence (Hernandez and Weiner 1986; de Vegvar et al,. 1986). A similar sequence has been found to direct correct 3' end formation also of the U2 snRNA (Hernandez, 1985). In this respect it has been suggested that these snRNAs must be transcribed by a specialized transcription machinery that differs from that synthesizing mRNAs. Recent work by Medlin et al. (2003) has shown that termination does not occur properly if the CTD of pol II has been deleted, indicating that factors required for 3' end formation are recruited very early during transcription. The region containing the U1 snRNA gene promoter extending from the BamHI site, at position -400, to the BglII site, at position -6 with respect to the initiation site, was cloned into the BamHI/NheI sites of the pSP65 plasmid through the use of a synthetic polylinker (Figure 1A). The resulting construct (psiUx) has a very strong promoter but lacks the transcription initiation site and the terminator. According to our design, these sequences should be provided by the inserted synthetic double stranded fragment, together with the siRNA target sequences. The initiation sequences is 5'-GATCTCA-3', where the last residue corresponds to the +1 nucleotide of the U1 snRNA (a G is also accepted in this position). The terminator element is 5'-CCCCTG'ACTTTCTGGAGTTTCAAAAGTAGAC-3' (SEQ ID NO:18), where the underlined sequence is the so-called 3' box, located 10 nucleotides downstream from the 3' end of the transcript. The CCCCTG sequence corresponds to the last 6 transcribed nucleotides of the U1 snRNA which have been shown to contribute to an efficient and site-specific 3' end formation (Hernandez, 1985). Cloning of the siRNA precursor sequence into psiUx can be performed very easily by inserting an amplified fragment or annealed synthetic oligonucleotides with ends compatible with the selected sites of the plasmid. While a 5' BglII terminus is obligatory since it is required to restore the initiation site, any of the sites contained in the polylinker (KpnI, XhoI, NheI and BamHI) can be utilized at the 3' end (Fig .1).